Antigenic studies on coronavirus. I. Identification of the structural antigens of human coronavirus, strain 229E[1]

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Antigenic analysis of human coronavirus, strain 229E (HCV/229E), using a microimmunodiffusion technique, has resulted in the detection of six virion antigens. Comparison of the effect of several different virus-disrupting agents has shown that sodium deoxycholate or Triton X-100 were the best for HCV/229E disruption. Of the six coronavirion antigens, three were identified as virus specific and the remainder as host antigens, which were present as either integrated or nonspecifically adsorbed host components. One of the virus-specific antigens was identified as the internal nucleoprotein (229E/RNP). On the basis of immunodiffusion reactions with isolated 229E/RNP it was concluded that human convalescent sera reacted specifically with the 229E/RNP antigen.

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L'analyse antigénique du coronavirus humain, souche 229E (HCV/229E), par la méthode de microimmunodiffusion a permis de déceler six antigènes virals. L'étude comparative des différents agents disruptifs a démontré que le déoxycholate de sodium ou le Triton X-100 étaient les meilleurs pour la rupture du HCV/229E. Des six antigènes du coronavirus, trois furent identifiés comme virus spécifiques et les autres comme antigènes cellulaires, lesquels étant des éléments soit intégrés ou non-spécifiquement absorbés. Un des antigènes spécifiques du virus fut identifié comme étant une protéine nucléique interne (229E/PRN). A partir des réactions d'immunodiffusion avec la protéine isolée 229E/PRN, il fut conclu que le sérum convalescent humain était spécifiquement réactif à l'antigène 229E/PRN.

Introduction

The coronaviruses have been classified as a separate group primarily on the basis of their distinctive morphology. Further supporting evidence for this classification depends mainly on the consistent characteristics associated with the genome RNA, i.e., linear, high molecular weight, single-stranded RNA, polyadenylated at the 3′ terminus, infectious, presumed of positive polarity. Information on the biochemistry of the viral polypeptides tends to vary according to the strains studied, with the exception of an internal polypeptide associated with the RNA, which, in all cases studied, appears to have a similar molecular weight of about 50 000 (Tyrrell *et al.* 1978; Robb and Bond 1979). Therefore, to provide a sound basis for the division of the accepted strains into possible subgroups and for the inclusion of new viruses with coronatypical morphology into the group, information on the antigenic and biochemical characteristics of coronaviruses is required.

Although coronaviruses might be expected to show antigenic relationships among members of the group, the available data tend to be confusing. Part of this confusion can be attributed to the variety of techniques used to study various members of the group and in part to the lack of a comprehensive study of these antigens in relation to their structural and biological functions. Additionally, the known incorporation of host components into the infectious virion (Hierholzer *et al.* 1972; Pike and Garwes 1977) makes the characterization of such antigens difficult, since any procedures designed to yield highly purified antigens may succeed simply in removing essential viral envelope along with nonvirion host components.

Relationships between coronaviruses have been studied serologically by hemagglutination inhibition, complement fixation (CF), neutralization, and immunodiffusion tests; varying degrees of cross reactions have been demonstrated between the human and animal viruses (reviewed by Robb and Bond 1979). Early studies have been reviewed extensively by McIntosh (1974). Since then, several studies using immune precipitation in agar gel for the detection of coronavirus antigens have been reported (Bohac *et al.* 1975; Bohac and Derbyshire 1976; Hajer and Storz 1978; Hierholzer 1976); however, the function of these antigens was not further identified with the exception of a host component identified by Hierholzer *et al.* (1972).

The aim of our studies has been to clarify these relationships. The first requirement was to fully characterize the antigens of one strain of coronavirus species

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by isolating and identifying the virion antigenic components with respect to their structural, morphological, and biological properties. These antigens would subsequently be compared with those of other coronaviruses to detect and identify the species and putative group-specific antigens. Human coronavirus, strain 229E (HCV/229E), was chosen for these investigations. In this paper, we report the results of our experiments to evaluate the effect of selected virus disruption procedures on the antigenic structure of the virus particle, to establish the full spectrum of coronavirus structural antigens detectable by the microimmunodiffusion technique in cellulose acetate, to determine the antigenic relationships of the virus structural components to the antigens of the host cell, and to identify the internal component antigen.

Using the sensitive cellulose acetate microimmunodiffusion technique (Johnson *et al.* 1964), six reacting virion components have been detected following disruption of purified virus with sodium deoxycholate or Triton X-100. Of these, three have been identified as virus-specific antigens, whereas three, which show dual virion and host specificities, have been classified as host components present either as an integral part of the virion or as nonspecific adsorbed host contaminant. One of the virus-specific antigens has been identified as the internal nucleoprotein (229E/RNP).

Materials and methods

Virus and cells

Cell culture, media, preparation, concentration, and purification of [^{3}H]uridine-labelled HCV/229E have been described previously (Kennedy and Johnson-Lussenburg 1976).

Antigens

Host antigen (L132)

This consisted of uninfected L132 cell monolayers washed three times with phosphate-buffered saline to remove fetal calf serum, extracted with minimal volumes (5 mL/75-cm^2 flask) of 0.001 *M* phosphate buffer, pH 7.2, by three freeze–thaw cycles, and clarified by centrifugation at 2000 rpm for 20 min at 4°C. The protein content of L132 antigen was 3.0 mg/mL.

Virus–host antigen (229E/L132)

This was obtained by similarly extracting virus-infected cell monolayers. These latter preparations were sometimes fortified by adding the washed and cushioned semipurified virus resulting from the concentration of infected cell lysates by ultracentrifugation. Final protein concentration of these preparations was 3–4 mg/mL.

Virus antigen (229E)

This consisted of purified virions concentrated approximately 300 times and suspended in 0.001 *M* phosphate buffer. A final protein concentration of 260–320 μg/mL was obtained.

Virus disruption procedures

All preparations were tested by immunodiffusion immediately following treatment with any of the agents described below.

Ether

Equal volumes of ether (Mallinckrodt Chemical Works) and virus antigen were incubated for 2 h at 37°C with occasional vigorous shaking. The two layers were allowed to separate and the ether layer removed by pasteur pipet. Residual ether was evaporated using a gentle stream of nitrogen or by exposure to air.

Chloroform

Chloroform was added to purified virus (1:1) and the mixture was swirled gently for 2 h at room temperature. Chloroform was then removed by evaporation at 37°C.

Trypsin

Trypsin (0.1 mL, 2.5%) (Grand Island Biological Company) was added to 1 mL purified virus and the mixture incubated at 37°C. At the end of 2 h of incubation, 0.05 mL soybean trypsin inhibitor (5%) was added to stop the reaction.

Pronase

Purified virus (0.2 mL) was treated with 20 μg pronase (Calbiochem) by incubating at 37°C for 6 h with intermittent agitation (Reginster 1966).

Triton X-100

A 10% solution of Triton X-100 (TX100) (Rohm and Haas Company) in distilled water was incubated with virus concentrate to a final detergent concentration of 1%. The mixture was incubated at room temperature for 2 h with frequent shaking.

Sodium deoxycholate

An aqueous solution of 10% DOC (Difco Laboratories) was added to purified virus concentrate to a final concentration of 1%. The mixture was then incubated for 2 h at room temperature.

Sarkosyl

A 20% aqueous solution of sarkosyl (SARK.) (Ciba-Geigy Foundation) was added to purified virus to a final concentration of 1% and the mixture incubated for 2 h at room temperature with frequent shaking.

Isolation of HCV/229E internal component (229E/RNP)

Disruption of the purified HCV/229E virus concentrate with Nonidet P-40 (Shell Chemicals) followed immediately by centrifugation to equilibrium on a sucrose density gradient according to the method previously described (Kennedy and Johnson-Lussenburg 1976) yielded [^{3}H]uridine-labelled virus ribonucleoprotein (RNP) with a density of 1.27 in sucrose. The protein concentration of the final product was 90–100 μg/mL.

Preparation of analytical antisera and ascitic fluids

Initial attempts to produce antibodies to HCV/229E in rabbits yielded very weakly reacting antiserum, confirming the observation of others (Bradburne 1970) that the rabbit was not a suitable animal for this purpose. The guinea pig was the animal of choice, but since large quantities of antiserum were required, a method for augmenting serum production with the induction of immune ascites similar to that used in mice was developed (Munoz 1957; Sommerville 1967). Recently, a similar procedure has been described by Stux *et al.* (1977). Male guinea pigs (Hartley/albino outbreed) weighing 400–

500 g were used. The animals received the first sensitizing dose by injection of 0.2 mL of the appropriate antigen in the footpad. Four days later, 1 mL of antigen mixed with complete Freund's adjuvant (1:1) (Gibco) was injected subcutaneously, followed at 2-week intervals by two intramuscular injections, each 1 mL, of the same antigen–adjuvant mixture. Then, after another 2-week interval, the ascitic fluid preparation was started with a 4-mL peritoneal injection of antigen – complete Freund's adjuvant mixture (1:1). These injections were continued weekly for 4 weeks. Fifty percent of the guinea pigs started to develop ascites after the second intraperitoneal injection, and those that did not were stimulated to do so by the third or fourth intraperitoneal injection. The fluid was tapped when distention of the abdominal cavity was noticeable, usually every 2 days. This was accomplished without anesthesia, using a 50-mL syringe equipped with an 18-gauge needle that was inserted into the lower left part of the abdomen. The fluid was transferred to 50-mL Corning disposable centrifuge tubes and left overnight at 4°C. The samples were then centrifuged at 3000 rpm for 20 min. An aliquot of the supernatant fluid was titrated to determine the concentration of antibody; the remainder was stored at −20°C.

The animals were bled before the administration of the antigen and weekly after each antigen injection by the heart puncture route. The blood was left to clot overnight at 4°C and the serum was removed after centrifugation at 2000 rpm for 20 min.

Reactivity of the serum and ascitic fluid was further increased either by ultrafiltration concentration using a PM 30 membrane in an Amicon ultrafiltration cell under nitrogen pressure in the cold room (4°C) or ammonium sulfate precipitation of gammaglobulins following standard batch procedures (Campbell *et al.* 1964).

Human convalescent sera

Through the courtesy of P. Phipps at the Regional Virus Laboratory of the Children's Hospital of Eastern Ontario, sera from patients with clinical respiratory illness were routinely screened by the CF test using a CF coronavirus antigen that we had prepared with HCV/229E grown in L132 cells (Hamre and Beem 1972). Positive sera, having titres that varied between 10–20 and 80, were provided for this investigation. These sera reflected the presence of low levels of antibody due to presumed natural coronavirus infection.

Protein estimation

Concentration of protein was estimated by the method of Lowry (Leggett-Bailey 1962) using bovine serum albumin as the standard.

SDS – polyacrylamide gel electrophoresis (SDS–PAGE)

Slab gel electrophoresis was carried out in 10.0% acrylamide, Tris–glycine buffered gels containing 0.1% SDS following the method described by Bingham (1975). Molecular weight standards used included bovine serum albumin, ovalbumin, myoglobin, lysozyme, transferrin, alcohol dehydrogenase, carbonic anhydrase, and cytochrome *c*.

Antiserum and ascitic fluid titration

Complement fixation

Again, through the courtesy of P. Phipps, CF titres of the antisera were obtained using 229E CF antigen supplied by us and prepared according to the method of Hamre and Beem (1972). The titre of the antigen was 8.7×10^7 plaque-forming units (PFU)/mL. Normal prebleed sera were included as controls and in all cases were found to be negative. All serum and ascitic fluid samples were titrated before concentration.

Neutralization of infectivity

The virus neutralizing ability was determined by plaque reduction. Twofold serial dilutions of antiserum or ascitic fluid were prepared in saline. Aliquots of 0.5 mL of each dilution were mixed with 0.5 mL of a suspension of HCV/229E in M199 (concentration ca. 500 PFU/mL). After 1 h of incubation at room temperature, 0.33 mL of the mixture was transferred to a L132 cell monolayer in a 75-cm^2 flask. Two monolayers were used for each dilution. After 1 h of adsorption at room temperature, during which time the inoculum was frequently redistributed over the monolayer, the agar overlay was added to the cells, which were then incubated at 33°C for 6–7 days for plaque development (Kennedy and Johnson-Lussenburg 1976). A virus control consisting of 0.5 mL virus diluent plus 0.5 mL saline was prepared from which a 0.33-mL sample was assayed. Also included was a cell control to monitor uninfected cell development. The highest dilution of serum or ascitic fluid showing 50% plaque reduction was considered the end point of the titration.

Immunodiffusion test

The microimmunodiffusion test in cellulose acetate, slightly modified by the use of stainless steel chromatography clips to hold the perspex template in place, was used (Johnson *et al.* 1964).

Results

The serological properties of the antisera and ascitic fluids were determined by complement fixation and virus neutralization titrations (Table 1). Following preliminary assessment of precipitating capacity in immunodiffusion reactions, collected antisera or ascitic fluids were pooled and concentrated by either ammonium sulfate precipitation or ultrafiltration before being

TABLE 1. Characteristics of immune fluids for identification of coronavirus antigens

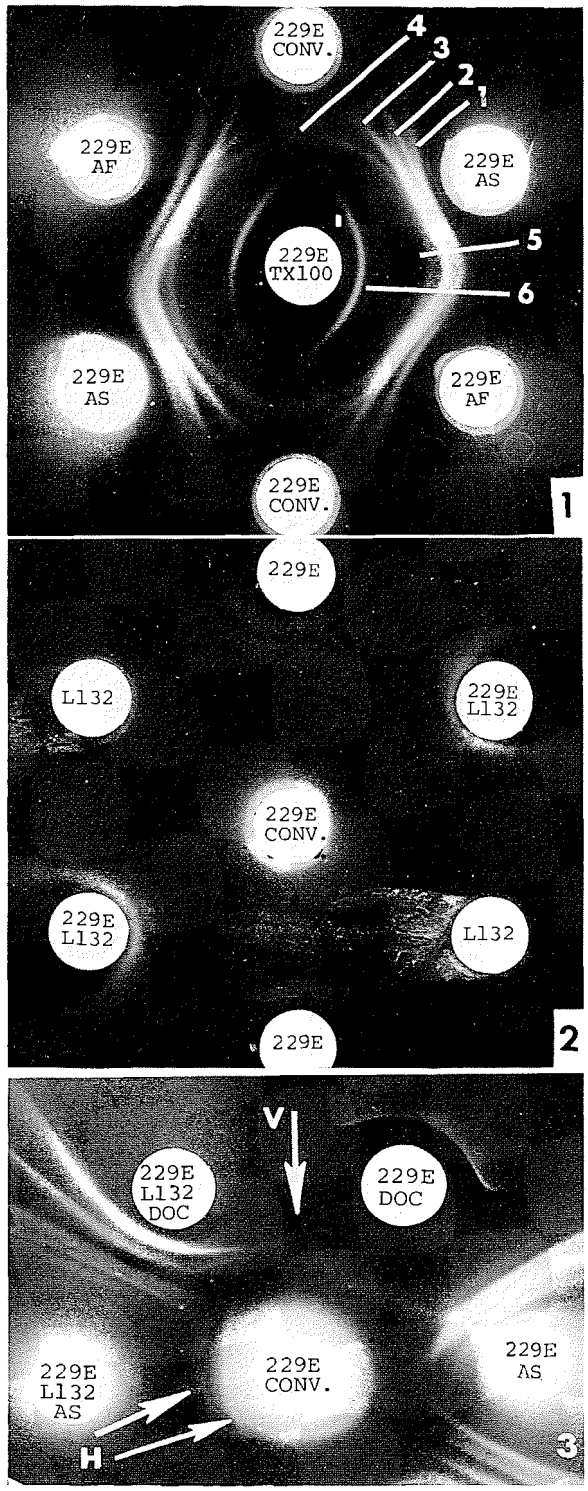
Antigen	Immune fluid*	Serological test	
		CF	Neutralization
Host extract	L132/AS	>320	0
	L132/AF	32	0
Virus–host extract	229E/L132/AS	1280	5120
	229E/L132/AF	>32	2560
Purified virus	229E/AS	640	5120
	229E/AF	>32	2560
Natural infection	229E/CONV. 1	10	80
	229E/CONV. 2	20	80
	229E/CONV. 3	80	40

*AS, guinea pig antiserum; AF, guinea pig ascitic fluid; CONV., human convalescent serum.

analyzed. Control immunodiffusion reactions demonstrated that both concentration procedures were equally effective. Several batches of each antigen were prepared and pooled as required to provide adequate amounts for use in this study.

The precipitating characteristics of each serum were determined using each antigen, and representative immunodiffusion reactions are shown in Figs. 1–3. Six virion components were revealed equally well by reaction with precipitins in either antiserum or ascitic fluid (Fig. 1) and at least one of these was detected by the human convalescent serum. The convalescent serum component was defined as antiviral because no precipitin line developed between the convalescent serum and the uninfected host antigen (Fig. 2), and because a reaction of identity developed between the reacting components of the specific antiviral reactions (Fig. 3). Thus, the convalescent serum was by definition capable of detecting coronavirus 229E virus-specific antigens.

A further complication occurred in some reactions, however, because of the human antigens shared by the host cells (L132) and the human serum, both of which were detectable by the antihost and anti-virus–host immune reagents (Fig. 3). These reacting components were classified as host specific and in some reactions (not shown) they formed patterns of nonidentity with the specific viral reacting components detected by the human convalescent serum. This finding provided addi-



ABBREVIATIONS: 229E, undisrupted, purified, and concentrated human coronavirus 229E antigen; 229E/DOC, purified, concentrated HCV/229E disrupted by sodium deoxycholate (DOC); 229E/TX100, purified, concentrated HCV/229E disrupted by Triton X-100; 229E/SARK., purified, concentrated HCV/229E disrupted by sarkosyl; 229E/L132/DOC, semipurified, concentrated HCV/229E antigen treated with DOC; 229E/RNP, internal component derived from purified HCV/229E; L132, uninfected cell extract consisting of only host antigens; 229E/AS and 229E/AF, guinea pig antiserum and ascitic fluid, respectively, against purified and concentrated HCV/229E; 229E/L132/AS and L132/AS, guinea pig antisera against infected and uninfected cell extracts; 229E/CONV., convalescent human serum obtained by CF screening of sera from patients with clinical respiratory illness.

FIG. 1. Immunodiffusion reactions demonstrating six virion components (1–6) revealed equally well by reaction with precipitins in both 229E antiserum or ascitic fluid. One of these (4) is also detected by human convalescent serum 229E/CONV. FIG. 2. Immunodiffusion reactions demonstrating that the human convalescent serum reacts with only 229E antigens and not with uninfected host control (L132). The streaking was the result of scratches made by burrs underneath the perspex template when it was slipped over the surface of the cellulose acetate. FIG.3. Immunodiffusion reaction demonstrating the specificity of the precipitins present in the human convalescent serum by the patterns of identity (V). A further reaction between host precipitins in 229E/L132/AS and human serum antigens (H) reflects the presence of common human antigens in the 229E/L132 extract.

TABLE 2. Summary of results of virus disruption

Disrupting agent	Maximum number of antigenic components detected by*				Quality of reactions
	L132/AS	229E/L132/AS	229E/AS	229E/CONV.	
Ether	1	2	2	0	Fair, well balanced
Chloroform	2	2	3	0	Poor, unbalanced
Trypsin	2	2	3	0	Fair, unbalanced, linkages poor
Pronase	1	1	2	0	Fair
DOC	4	5–6	6	1–3	Good, well balanced
TX100	2	4	6	1	Good, well balanced
Sarkosyl	ND	ND	3	1	Poor, diffuse

*Abbreviations as defined in Table 1. ND, not determined.

tional support for the classification of the convalescent serum as anti-HCV/229E specific.

Evaluation of virus disruption procedures

The disrupting agents were chosen according to their selective mode of action as well as for their historical significance in the study of viruses, and included lipid solvents (ether and chloroform), enzymatic degradation (trypsin and pronase), and surface active agents (DOC, TX100, and SARK.). SDS was not used because of known problems with nonspecific reactions in immunodiffusion tests (Johnson and Westwood 1971). The efficiency of the disruption of the virus particle was evaluated on the basis of (*i*) the number of antigenic components detectable after each treatment, (*ii*) the intensity and resolution of the immunodiffusion reactions of the released antigens with specific antisera, and (*iii*) the relationship of the reacting components to those in a standard reaction using DOC-disrupted antigen.

All the disruption treatments were performed on antigen preparations from the same pool. The results of the immunodiffusion reactions have been summarized in Table 2. It is clear that of the seven reagents tested, DOC and TX100 were the disrupting agents of choice, with DOC slightly favoured because of the better resolution and greater number of precipitating components in the reaction with 229E convalescent serum (Fig. 4).

Further analytical reactions were designed to compare the components released by the different treatments with those released by DOC. In most cases, it was found that reactions of identity developed between the released reacting components, and in no case was a reaction of nonidentity evident. A representative immunodiffusion reaction comparing the antigenic relationships between the components released by three surface active agents (DOC, TX100, and SARK.) as revealed by reactions with antiviral serum (229E/AS) and virus-specific convalescent serum (229E/CONV.) is presented in Fig. 4.

In this reaction, on the basis of precipitin lines developed with the convalescent and antiviral sera, more reacting components were detectable following DOC and TX100 virus disruption than after sarkosyl treatment. The nature of the extra component released by TX100 as revealed by reaction with antiviral serum (arrows) is not clear. On the one hand, in this reaction with 229E/AS, it does not appear to be released by DOC disruption; however, in the reaction with convalescent antiserum, there is a faint line that seems to link in a pattern of identity with the TX100-released antigen. In the reaction to clarify the relationship between the DOC- and TX100-released antigens (Fig. 5), the formation of two spurs (arrow) indicates the presence of two components showing a partial identity with the DOC-released antigen. Whether these components were lost by DOC treatment or are not detectable because they were not at optimal proportions or remain masked is not indicated. It is evident, however, that both DOC and TX100 are the disrupting agents of choice. Therefore, on the basis of these results, both DOC and TX100 should be used in analytical tests, DOC being of choice where the internal component is under study, and TX100 when the total number of components and their identity is of interest.

Identification of virus structural components

The reaction design used to identify the virus structural components is presented in Fig. 6. In this reaction, five of the total six components detectable can be seen. Numbered as in Fig. 1, component 5 has not been resolved. By virtue of the reaction of nonidentity with the host precipitin lines, component 4 is identified as virus specific. Components 1 and 2 also appeared to be associated only with the virus reaction and are classified provisionally as "V" antigens. The remainder were

detected equally well by both host and virus antiserum and are classified as host-specific components (H3 and H6), which are present either as an integral part of the virion or as adsorbed cell contaminant.

Identification of the HCV/229E internal component (RNP) antigen

The HCV/229E ribonucleoprotein (229E/RNP) was prepared by treatment of purified virus with Nonidet P40 following the procedure described (Kennedy and Johnson-Lussenburg 1976). SDS–PAGE analysis of the isolated product demonstrated that it was a single polypeptide with a molecular weight of ca. 50 000 with the same migration characteristics as the major protein component of the complete virion (Fig. 7). Radiolabelling experiments have shown that these polypeptides are not glycosylated (data not shown).

The 229E/RNP antigen was identified as a specific virion structural antigen by the reaction of identity with one of the TX100-released viral components (Fig. 8). In addition, this same precipitin line was deflected to form a weak pattern of identity (Johnson and Westwood 1971) with the faint reaction of the specific antiviral convalescent serum (Fig. 8). This identity was further confirmed by the reaction shown in Fig. 9 demonstrating that one of the viral precipitins present in the HCV convalescent serum was specifically anti-HCV/RNP. The occurrence of the second band in reaction with the concentrated 229E/AS is felt to be due to the prozone phenomenon sometimes seen in these reactions. This interpretation is supported by, first, the single band formed in the reaction shown in Fig. 8, and second, by the hazy convergence, especially in the area of the top well of the reaction.

As a result of these reactions, therefore, the human HCV/229E convalescent serum has been characterized as a valuable immunological reagent for the identification of the HCV internal ribonucleoprotein antigen. Further comparative immunodiffusion studies, using convalescent sera from human and other animal species in homologous and heterologous reactions against the indiginous strains of coronaviruses, could be expected to provide evidence for the identification of the coronavirus internal RNP component as the putative group-

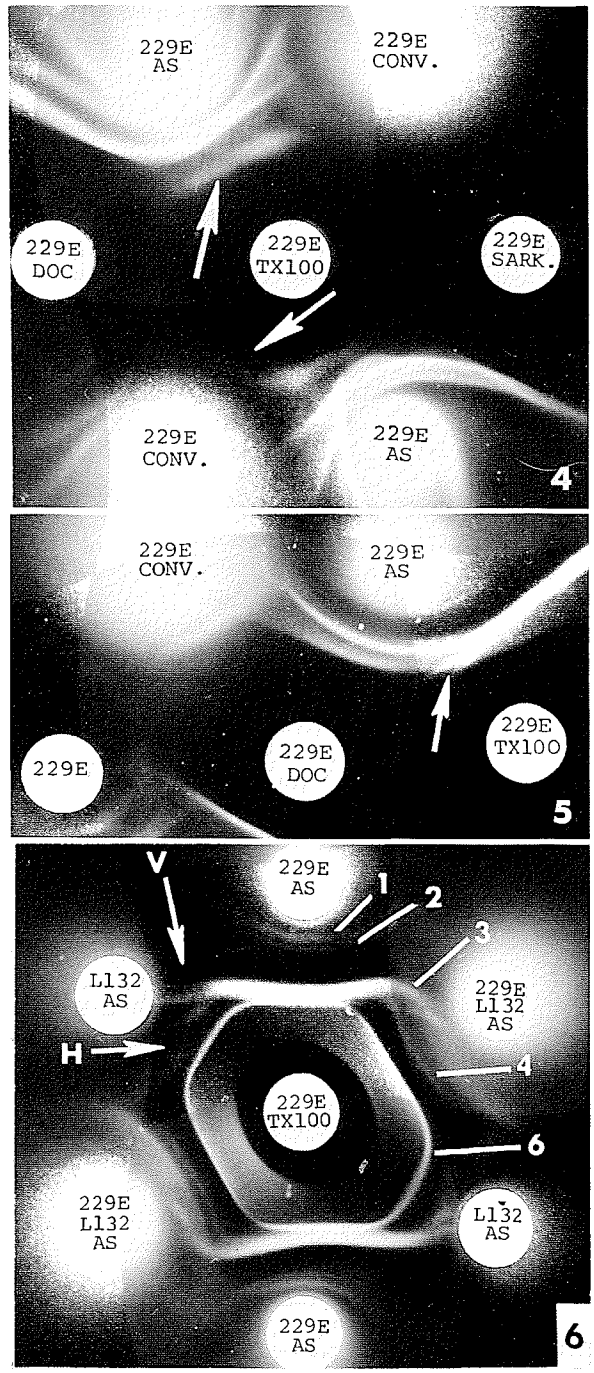


FIG. 4. Immunodiffusion reaction comparing the virion components released by treatment with DOC, TX100, and SARK. An extra component is revealed by TX100 treatment (arrows) but more components released by DOC treatment are detected by human convalescent serum. Only one faintly reacting component is evident following SARK. treatment. FIG. 5. The extra TX100 component forms two spurs in a further comparison of virion components released by DOC and TX100 indicating partial identity with the DOC-released antigens. The requirement for virion disruption is illustrated by the lack of reaction between the undisrupted virion antigen (229E) and human convalescent serum. FIG. 6. Immunodiffusion reaction using analytical sera for the identification of virion structural components released by TX100. Three (V, 1, and 2) are identified as 229E specific, whereas the remaining two are identified as host (H) specific.

specific antigen. The results of these studies will be reported in another communication (in preparation).

Discussion

This study was undertaken as a preliminary step in the comparative analysis of different coronavirus species in an attempt to clarify the confusing pattern of antigenic relationships reported for this group and to identify the putative group-specific antigen. Such a study must rely upon the ability to distinguish between host and virion structural antigens, but since host antigens are known to be incorporated in the coronavirion (Hierholzer *et al.* 1972; Pike and Garwes 1977), the approach to this problem demanded a method capable of not only detecting all the antigenic components but of analyzing their relationships as well. The immunodiffusion technique is ideal for this purpose and the characterization of both the analytic immune fluids according to their precipitin specificities and the reference virus preparation with respect to its full complement of antigenic components has been accomplished.

To ensure that all the virion antigens were available to participate in the immunodiffusion reactions with the different homologous immune fluids, it was necessary to release them by disrupting the virion. We tested seven different agents that could be grouped according to their selective mode of action as lipid solvents, degrading enzymes, and surface active agents. The selection of the most appropriate disrupting agent(s) was then made on the basis of the efficiency of the disruption of the virus particles as judged by (*i*) the number of antigenic components consistently detectable after each treatment, (*ii*) the intensity and resolution of the released antigens with specific antisera, and (*iii*) the relationship of the reacting components to those in a standard reaction. Previous work had shown that DOC was one of the most reliable agents for the release of influenza virus antigens (Johnson and Westwood 1971); therefore, DOC-disrupted coronavirus was used as the standard in the first reactions.

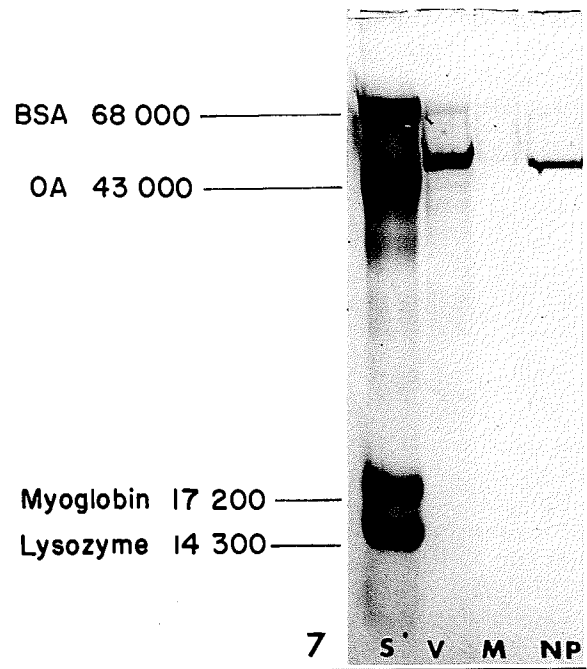


FIG. 7. SDS–PAGE of whole purified 229E virus (V, lane 2), virion internal component (NP, lane 4) isolated by density gradient centrifugation following treatment with Nonidet P40, and the membrane fraction (M, lane 3) from the same density gradient. Migrations of appropriate standards (S) are shown in lane 1. The NP polypeptide, with an approximate molecular weight of 50 000, is the major virion component, is present in all three preparations, but is the only polypeptide detectable in lane 4. Gels were stained with 1% Coomassie blue. BSA, bovine serum albumin; OA, ovalbumin.

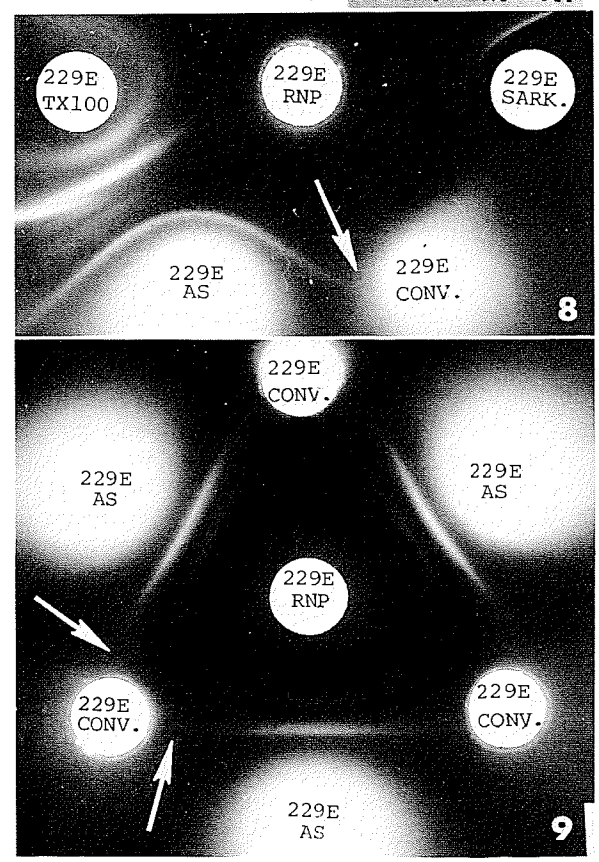


FIG. 8. Immunodiffusion reaction demonstrating the specificity of the internal component 229E/RNP by the reaction of identity with one of the TX100-released virion antigens. The deflection of the precipitin line (arrow) by the human convalescent serum indicates the presence of precipitins against the internal component. FIG. 9. Immunodiffusion reaction demonstrating by the reactions of identity (arrows) that the human convalescent serum is capable of identifying the internal component 229E/RNP.

As a result of these analyses, DOC and TX100 were shown to be the disrupting agents of choice (Table 2) and six reacting components were usually revealed in purified virus preparations and identified as virion structural antigens. The classification of these antigens according to their virus or host relationships was based on the development of immunodiffusion patterns of identity in comparative reactions. Thus, three reacting components were classified as virion specific and three as host specific. Whether the host antigens could be regarded as specifically integrated components essential for virion infectivity or as nonspecifically adsorbed contaminants remains to be seen. At present, however, we think that at least one viral antigen, appearing as a major precipitin line, was host derived and was most probably associated with the viral membrane. Support for this conclusion lies in the fact that, even after exhaustive purification, this component was still detectable in the same relative proportion to the other reaction components (unpublished data).

In immunodiffusion studies reported by others (McIntosh 1974; Robb and Bond 1979), at least one major viral antigen has been consistently described, often accompanied with one or two additional distinct antigenic molecules. However, a direct correlation between those antigens and the 229E virus structural antigens identified in the present study cannot be made for several reasons. First, the use of cellulose acetate membrane instead of agar as the matrix for immunodiffusion permits the resolution of a greater number of reacting components, and being inert precludes the possibility of nonspecific interaction between some of the test reactants and the agar (Crowle 1971). Secondly, in the early studies, either soluble antigens or nondisrupted virion antigens were examined. In the former case, the identity of the soluble antigens was not determined, whereas in the latter case, diffusion of a large membraned virus such as coronavirus is questionable and, therefore, reactions depended on the spontaneous disruption of intact virions in agar in the presence of antibody diffused from the neighbouring wells (Johnson and Westwood 1968). Third, though a number of antigenic studies have been carried out, they have not all been on the same strain of coronavirus. Therefore, the six virion structural components and the analytical immune fluids used in their identification must form the basis for further comparative analyses to clarify the relationships between antigens derived from different strains of coronaviruses and to identify any common group antigens.

With regard to the possible existence of group-specific antigens, since the number of polypeptides reported for different strains of coronaviruses is variable (Robb and Bond 1979), there seems to be no reason to expect a close correlation between their antigens. However, the polypeptide associated with the internal component of all strains of coronaviruses so far studied shows a strong similarity. It is the major virion polypeptide, is nonglycosylated, and by SDS–PAGE analysis has a molecular weight of ca. 50 000. It was therefore considered to be the most likely candidate to possess group antigenic characteristics. But in order to demonstrate such a group antigen, it was necessary first to establish a reference immunodiffusion reaction between the HCV/229E internal component and its specific antiserum. As the last step in this preliminary study, this was achieved by using HCV/229E/RNP released by Nonidet P40 and isolated on a sucrose-density gradient (Kennedy and Johnson-Lussenburg 1976) in reactions with the specific precipitins occurring in human convalescent serum.

In further antigenic studies, these HCV/229E structural antigens and the homologous immune sera will be used in comparative immunodiffusion reactions to determine the pattern of relationships between different species of coronaviruses. The anticipated identification of a specific coronavirus group antigen will provide a valuable criterion for the classification of the coronavirus group.

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